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Synthetic studies towards the luzopeptins: New amino acid synthons through the aza-Achmatowicz reaction

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Rice University, 1992
RICE UNIVERSITY

SYNTHETIC STUDIES TOWARDS THE LUZOPePTINS: NEW AMINO ACID SYNTHONS THROUGH THE AZA-ACHMATOWICZ REACTION

BY

TOSHIO SHIMIZU

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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APPROVED, THESIS COMMITTEE

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Houston, Texas
August, 1991
ABSTRACT

Synthetic Studies Towards the Luzopeptins:
New Aminoacid Synthons Through the Aza-Achmatowicz Reaction

by

Toshio Shimizu

A new method for the enantioselective synthesis of unusual amino acids has been
developed. The chemoenzymatic aza–Achmatowicz rearrangement of appropriate
furylglycine derivatives provided nitrogenous synthons that were readily converted to
amino acid building blocks. An application of the new chemistry to the synthesis of the
unique pyridazine carboxylic acid component of luzopeptins is presented. Luzopeptin C
has potent inhibitory activity towards the reverse transcriptase of HIV as the causative agent
of AIDS.
Acknowledgements

I would like to thank my advisor, Dr. Marco Ciufolini, for teaching me a lot of interesting details of Organic Chemistry. His endless patience and encouragement for me have let me know the pleasure of learning about Chemistry and making success for the achievement of new reactions. I had a lot of fun and the most exciting time in my life for 2 years in Houston with his help.

Many thanks to my lab partners, Mike Bishop, his wisdom and wit have always made me happy and I was able to do a lot of interesting and exciting things in U.S.A. with his help.

Also thanks to Dr. Norman Byrne, his amazing good sense of humor is one of the greatest memories for my American life.

To Angelica, Sandra and Melissa, thank you very much for your kindness and smile. I love your singing and dancing (also good natures!).

Finally, to all of my co-workers of Cosmo Oil Company, Japan, thank you very much for your kindness.
Synthetic Studies Towards the Luzopeptins: New Aminoacid Synthons
Through the Aza-Achmatowicz Reaction

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I. INTRODUCTION

Acquired immunodeficiency syndrome, or AIDS, has recently established itself as an extremely serious threat to public health. The most striking symptoms of the illness are unchecked proliferation of pathogenic bacteria and fungi within infected individuals, as well as the appearance of rare tumors, which eventually kill the patient. Contact with the blood of AIDS patients is likely to transmit the disease to a healthy person. Because of this, AIDS has rapidly spread to the population at large, transmitted by sexual contact, by sharing of needles among intravenous drug abusers, and, in some tragic cases, by transfusion of infected blood, or by accidental contact with infected blood by health care personnel.

The economic impact predicted to result from spread of the disease among the general population is staggering. The combination of lowered productivity because of illness among infected workers and the strains placed by the millions of individuals who are likely to develop full-blown AIDS during the next 5 - 10 years on the health care system, may cause financial losses totaling hundreds of billions of U.S. dollars. It is not surprising that a massive research effort has been mounted worldwide to control the new disease.

Recent evidence has implicated a human retrovirus (Human Immunodeficiency Virus, "HIV") as the causative agent of AIDS.¹ Unlike other human retroviruses,² which

appear to induce monoclonal transformations, HIV produces disease solely as a result of its *multiplication*. Replication of HIV, like that of all retroviruses, is dependent on a DNA polymerase, termed reverse transcriptase (RT), which is carried into the host cell by the virus itself. The RT, using the viral RNA genome as its template, controls the assembly of a viral DNA once the virus has penetrated its target cell. Viral DNA is eventually incorporated into the genetic material of the host cell. There, it remains dormant until unknown events trigger its sudden expression, namely, the production of new viruses within the cell. The rapid rate of viral replication kills the host cell, which soon breaks up releasing the new viruses. These may then infect additional healthy cells (Scheme 1).

![Scheme 1](image)


It is known that there are two types of HIV. One of these, termed HIV-1, is believed to be responsible for the AIDS epidemic that has spread all over the world. The second type, called HIV-2, has spread mainly among West African people, and it is less severe and not as regularly lethal as HIV-1. In this thesis, "HIV" represents HIV-1.

HIV shows special affinity for T4 lymphocytes, white blood cells that play a major role in the regulation of the immune system. The destruction of T4 cells causes severe immunodeficiency.

It is clear that inhibition of HIV-RT will block viral replication, and since RT is strictly a retroviral enzyme, it is a very logical target for antiretroviral therapy. Deactivation of RT would in fact prevent transcription of the viral RNA genome into a DNA strand, thus blocking all channels of communication between the invading virus and its host cell. One of the most effective resources for the treatments for AIDS is 2,3-dideoxy-3-azidothymidine (AZT), a nucleotide mimic which is readily phosphorylated in vivo, and which may subsequently be incorporated by RT into a growing strand of DNA. Attachment of AZT to an emerging DNA segment clearly precludes further chain growth, because no nucleophile is now available to establish a further 3'-5' phosphate linkage (Scheme 2). The stunted DNA fragment appears to remain tightly bound to the RT/viral RNA complex. RT thus ceases to function. The macroscopic consequence of this is a powerful inhibition (ID$_{50}$ = 0.04 µM) of that enzyme.

---


Scheme 2

The success of AZT-based treatments is overshadowed by the toxic side effects elicited in many patients. Furthermore, AZT-resistant strains of HIV have recently appeared,\textsuperscript{11} casting serious doubts about the long-term viability of the drug. A systematic search for novel anti-AIDS agents is therefore urgently needed.

Potent inhibitory activity towards RT has recently been discovered in Luzopeptin C,\textsuperscript{212,13} Most significantly, 2 to 5 µg/ml of 2 suppresses HIV replication in infected MT-


4 cells without toxic side effects. Indeed, the toxicity of luzopeptins is relatively low in vivo. Luzopeptin is a dimeric, cyclic decadepsipeptide. Three of its amino acids are common (glycine, sarcosine, D-serine), while quinaldic acid, 3, and N-methyl-3-hydroxyvaline, 4, are quite unusual. A final component, 5, is unique. We shall use the acronym "PCA" (pyridazine carboxylic acid) when referring to 5. It is obvious that once all of the amino acid components of 2 are available, a synthesis of luzopeptins will become possible (Scheme 3).

Scheme 3

The mechanism of action of 2 remains a mystery. Luzopeptins are quite rare substances, so that an effort to clarify the anti-AIDS potential of these antibiotics must rely heavily on totally synthetic materials. A rough picture of the structure-activity relationship of luzopeptin C is emerging and already suggests that only a portion of the molecule is responsible for anti-RT (and, presumably, anti-HIV) action. Of course, *in vitro* activity against RT does not necessary imply antiviral potency *in vivo*, but SAR studies should help to clarify the issue. The ID₅₀ of 2 towards RT has not been expressly stated, however, it may be estimated to be around 1 μg/ml.¹¹ On a molar basis, 2 is therefore about 10-20 times less potent than AZT. However, since only a portion of the molecule is likely to be responsible for activity, one should be able to determine such "minimal active substructure," to amplify its antiretroviral potency, and to develop a new generation of antiretroviral agents with activity equal to, or better than, 2. The success of such studies depends on the availability of synthetic 2, and analogues thereof, and of reliable chemical methodology for the preparation of the requisite substances.
II. BACKGROUND

No total synthesis of any member of the luzopeptin family of antibiotics has been described to date. Yet, a synthetic route to 2 appears to be a prime requirement for the feasibility of further SAR and pharmacology studies, especially because luzopeptins are extremely rare. Three of their component amino acids, glycine, sarcosine, and D-serine are commercially available. The quinaldic acid fragment, 3, may be obtained from the native antibiotic by chemical degradation, but because of difficulties in securing the natural product, a synthesis of 3 is necessary. By contrast, N-methyl-3-hydroxyvaline and PCA do not survive degradation. A synthetic attack on 2 must clearly deal with three potentially troublesome issues: technology for the formation of the macrocycle, preparation of N-methyl-3-hydroxyvaline in optically active form, and, most critically, the asymmetric total synthesis of PCA.

Macrocycle formation in systems related to 2 was examined in 1986 by Olsen and collaborators (Scheme 4). These workers described the cyclization of 2 by peptide bond formation between sarcosine (COOH terminus) and valine.\textsuperscript{15} This reaction proceeded in a 66 % yield.

\begin{center}
\includegraphics[width=\textwidth]{scheme4.png}
\end{center}

\textbf{Scheme 4}

Earlier work from this group established methodology for the synthesis of optically active 3-hydroxy-N-methyl valine, 4,\textsuperscript{16} and for its incorporation into a model tripeptide corresponding to the lower sector of 2.\textsuperscript{17} (Scheme 5).

Scheme 5


The synthesis of PCA constitutes the main goal of our work. Saturated versions of PCA such as 9 are found as components of certain fungal metabolites of current interest, and are termed piperazic acids (Figure 1). The preparation of these substances has been studied extensively by Hassall, in connection with work on the monamycin group of antibiotics. However, synthetic schemes developed for piperazic acids are inadequate for the preparation of unsaturated analogues.

\[
\text{OH} \hspace{1cm} \text{COOH}
\]

9

Figure 1

A retrosynthetic analysis of the molecule suggested that ring formation may be effected by cyclization of an aldo-hydrazone acid such as 10 (Scheme 6). An important issue implicit in the preparation of this advanced intermediate relates to the control of stereochemistry at the two adjacent stereogenic carbons. Technologies based on introduction of the hydrazone group\(^{20}\) through the agency of the enolate of 11 seemed unsuitable, because of anticipated difficulties with \(\beta\)-elimination of the oxygen functionality. Likewise, aldol condensation between a protected dialdehyde and a glycine enolate equivalent\(^{21}\) seemed inappropriate. The aldol product would form as the syn


isomer, necessitating a later inversion of configuration at a site prone to undergo elimination, because of its location $\beta$ to a carbonyl group.

\[
\begin{align*}
\text{z} & \quad \text{w} & \quad \text{x} \\
\text{5} & \quad \text{10} & \quad \text{11}
\end{align*}
\]

Scheme 6

A total synthesis of PCA reported by Hughes and Clardy$^{22}$ during the course of our own studies underscores some of the foregoing themes (Scheme 7). Those investigators took advantage of the favorable (and unexpected)$^{23}$ propensity of epoxide 12 to react with hydrazine to furnish 13 selectively. Compound 13 was obtained in optically active form through the agency of a key Sharpless epoxidation. Established chemistry was used to complete the synthesis.

\[
\begin{align*}
\text{z} & \quad \text{w} & \quad \text{x} \\
\text{12} & \quad \text{1. RuO}_4 & \quad 2. \text{CH}_2\text{N}_2 \\
\text{13} & \quad \text{1. Aq. K}_2\text{CO}_3 & \quad \text{MeOH} \\
\text{1. H}_2\text{N-NH}_2 & \quad \text{2. } \text{H}_2\text{N-NH}_2 & \quad \text{K}^+ \cdot \text{COOC} \\
\text{13} & \quad \text{MeOOC} & \quad \text{OH} \\
\text{13} & \quad \text{MeOOC} & \quad \text{OH}
\end{align*}
\]

Scheme 7


Our plan, however, envisioned reaching PCA starting from 15, which is readily available via aza-Achmatowicz chemistry. The aza-Achmatowicz reaction is defined as the rearrangement of furfurylic amides to tetrahydro β-piperidones (cf. 14 → 15). Earlier work from these laboratories defined a chemoenzymatic protocol whereby racemic furylglycine derivatives, readily available in hundred-gram lots through efficient chemistry, are converted into oxazolone 15. Compound 15 is a versatile synthetic intermediate that may serve as building block for the synthesis of piperidine and izidine alkaloids, azasaccharides, carbacephams, etc.

Oxazolone 15 seemed an ideal starting compound for the synthesis of unusual amino acids. Our ability to reduce the ketone in a stereospecific sense translates into the accessibility of either syn or anti stereoisomer of aminoalcohol 16. Cleavage of the carbamate and oxidation of the primary OH to an acid, with or without opening of the piperidine ring, would deliver 17 or 18, from which a host of nonprotein amino acids might be synthesized (Scheme 8).

Scheme 8

24This work is not yet made public.
The main objective of the present work was to investigate a protocol whereby the foregoing surmise might be reduced to practice, particularly in connection with the preparation of acyclic amino acid synths.
III. DISCUSSION

*New Amino Acid Synthons Through the Aza-Achmatowicz Reaction*

Acyclic building blocks were envisioned to arise from 15-a and 15-b through ozonolysis of enamides 21-a and 21-b. These are readily obtained from 15 as shown in Scheme 9 below.

![Scheme 9](image)

**Scheme 9**

Aza-Achmatowicz enamides undergo a number of reactions of great synthetic usefulness. A trasformation that we examined briefly as an interesting aside during our involvement in PCA synthesis was the conjugate addition of the moderately nucleophilic double bond of 21 to conjugated carbonyl receptors. This novel reaction was found to take place under activation by Lewis acids, BF₃·OEt₂ being particularly successful. Unfortunately, it proceeded only in modest yields, and, at that, only with particular receptors. For instance, addition of a solution of enamide 21 to the complex of methyl vinyl ketone with BF₃·OEt₂ provided 22 in 20 % chromatographed yield (Scheme 10). Compounds of the type 22 should be very useful in organic synthesis. Perhaps future

---

research will identify a suitable combination of Lewis acid, solvent, and reaction conditions that might render this Michael-like addition more general and efficient.

Scheme 10

The double bond of enamide 21 was found to undergo ozonolysis in a normal fashion. The presumed intermediate aldehydes may be trapped, e.g. with a Wittig reagent, but, the yields of such reactions tend to be low. A close examination of this chemistry led us to conclude that survival of intermediate 23 was compromised by a facile β-elimination of acetic acid (Scheme 11).

Scheme 11

It was felt that such problems might be resolved, or at least attenuated, if the OH protecting group were an ether instead of an ester. An ether could only decompose with ejection of an alkoxide, a much poorer leaving group than a carboxylate. Consequently, the elimination shown above may be considerably retarded. A search for a more suitable blocking group was therefore launched, and racemic materials were initially utilized in these

26Hermann, C. Y. W. Dissertation; Rice University, 1990.
efforts. It was observed that while acetylation of alcohols 19 was clean and efficient, this was not the case for benzylolation. Thus, treatment with NaH / BnBr in DMF or THF produced only 10 - 15 % of the desired benzyl ether 24 (Scheme 12), while the balance of the starting material was converted to a complex mixture of products. Similarly, reaction of the alcohols with MOM-Cl and Hüning base proceeded poorly.

Scheme 12

Fortunately, it was observed that an exchange of protecting groups could be effected at the stage of enamide 21. Subjection of the latter to MeOH / K₂CO₃, followed by reprotection with a MOM group, afforded 26 in excellent yields (Scheme 13).
Ozonolysis of 26 proved to be much cleaner than the analogous reaction in the acetate series. Still, isolation and purification of the aldehyde was plagued by major losses of material. A substantial improvement in the efficiency of this process was achieved by inducing dimethyl acetal formation from aldehyde 27 in situ, under Luche conditions. This reaction is particularly advantageous, since the ozonolysis reaction itself is carried out in methanolic medium. Thus, upon completion of the ozonolysis and reduction of peroxidic material (Me₂S), cerous chloride heptahydrate was added to the crude reaction mixture and acetal 28 was obtained directly. Presumably, the N-formyl group in the primary product of ozonolysis is transferred to MeOH during acetal formation, however, no efforts were made to verify the presence of methyl formate in the crude reaction mixture. Purification by column chromatography afforded 28-a in 60.0 % overall yield. In an analogous fashion, 28-b was obtained from 26-b (Scheme 14).

![Scheme 14](image)

Cleavage of the oxazolone was studied next, in preparation for oxidation of the intermediate alcohol to an acid. It was observed that whereas base hydrolysis of 28 readily afforded amino alcohol 29, which subsequently may be selectively N-protected to afford derivatives 30 - 32, a Kunieda-type sequence\(^{28}\) was particularly advantageous for our purpose. Reaction of 28 with BOC\(_2\)O produced 33, which was rapidly cleaved to 31 using methanolic K\(_2\)CO\(_3\) (Scheme 15). The ring-cleaved product is thus formed in protected form, facilitating extraction and purification of this otherwise extremely polar material to a considerable extent.

Oxidation of the alcohols thus obtained was found to proceed particularly well under the Garner condition (basic KMnO₄). However, the CBZ-protected compound 30 and TROC derivative 32 were poor substrates for this reaction. The CBZ derivative suffered from a competing oxidation of the benzylic methylene to a carbonyl. By contrast, the TROC protected compound 32, was found to undergo rapid cyclization back to oxazolone 28 under the basic conditions of the Garner oxidation.

Amino acid 34 is rather sensitive, since a carboxylic group and an acetal coexist in the same molecule, consequently, it was immediately esterified (CH₂N₂). Chromatography afforded pure methyl ester in 50% yield (Scheme 16).

The chemistry discussed above was repeated using optically active 26-a, [α]D²⁵ = +93.5° (c=0.515; EtOH) and 26-b, [α]D²⁵ = −30.0° (c=0.924; EtOH). From each MOM enamides, the optically active compounds, 35-a, [α]D²⁵ = −22.3°(c=0.520; EtOH) and 35-b, [α]D²⁵ = +20.7°(c=0.492; EtOH) were obtained. These transformations demonstrate chemoenzymatic aza-Achmatowicz methods may be utilized for the synthesis of amino acids.

Efforts Towards PCA

Conversion of 28 to PCA requires conversion of its amino functionality to a hydrazine. Two routes were studied in order to achieve the desired transformation: direct amination of the oxazolone, and a Hoffman-type rearrangement of a urea derivative.

Some O-derivatives of hydroxylamine, such as the O-sulfonic acid,\textsuperscript{30} are well established synthons for the fragment "NH$_2^+$". It seemed logical to explore the possibility of inducing a direct amination of the N-anion of oxazolone 28 with such reagents. Hydroxylamine O-sulfonic acid proved to be entirely unsatisfactory, because of solubility problems. By contrast, the Klotzer-Harger reagent,\textsuperscript{31} O-diphenylphosphinyl hydroxylamine, effected clean N-amination of the sodium salt of 28 (NaH) in DMF. Curiously, this reaction failed with acyclic analogues of 28, presumably because of steric reasons. Compound 36 is sensitive and very polar. Consequently, it was treated directly with 1 N HCl, which induced conversion into tetrahydropyridazine 37 in 73.1 % yield (Scheme 17). Cyclization could also be achieved by refluxing an acetonitrile solution of 36 in the presence of a catalytic amount of quinolinium camphorsulfonate (QCS), but this second protocol was not as efficient. It appeared that the higher temperature necessary for the QCS-promoted reaction, and the longer contact time, caused formation of substantial quantities of polar byproducts.


Scheme 17

The oxazolone ring of 37 underwent smooth and rapid hydrolysis with 0.1N NaOH at room temperature (98.8%) (Scheme 18). This is remarkable for an oxazolone, which normally requires much harsher conditions. We presume that the N-anion of 38 is formed as an intermediate during break-up of the carbamate. The fact that this anion is resonance-stabilized, and therefore not as basic as an ordinary N-anion, presumably facilitates cleavage of the oxazolone. Compound 38 is water soluble, and it cannot be extracted into common organic solvents. Indeed, 38 could only be recovered by neutralization of the hydrolysis solution (Amberlite IRC-50 resin), followed by freeze-drying and extraction of the solid residue with acetone. These solubility properties seriously hampered further manipulations: conversion of 38 to an appropriate derivative had become highly desirable.

Scheme 18
Studies directed towards the protection of 38 allowed us to investigate the acylation chemistry of the hydrazone. A matter of considerable concern was whether free PCA may be introduced directly into a polypeptide chain, i.e. N-acylated regioselectively at the amino, rather than the imino, N atom, and in the usual fashion (DCC, etc.). Because of the hydrazone nature of PCA, unusual reactivity may be observed. Surprisingly, we were unable to find any literature about the chemistry of dihydropyridazines of the type 38. It was known that secondary hydrazones and dihydropyrazoles (i.e. five-membered ring analogs of our compounds) undergo regiospecific acylation at the amino nitrogen, and that a potentially calamitous C-acylation (i.e. enamine-like reactivity) is not to be expected.\textsuperscript{32} Still, the possibility of exceptional reactivity could not be ruled out at the onset of our investigation. Fortunately, acylation of 38 occurred in the desired fashion. Even treatment with excess acetic anhydride in pyridine caused no attack at the imino nitrogen, and consequent tautomerization to a diacyl enehydrazide. Of particular significance is the clean reaction of 38 with p-nitrophenyl octanoate, which furnished derivative 39 in 60.6\% yield (Scheme 19). Since p-nitrophenyl esters are excellent carriers of activated α-aminoacyl groups for peptide synthesis, it seems plausible that later construction of a PCA-containing peptide might be carried out in a similar fashion. The newly formed octanamide derivative was still water-soluble, but it was nicely extractable into various organic solvents, facilitating subsequent work-ups to a significant extent. The desired PCA now appeared to be just an oxidation step away from 39.

Oxidation of 39 turned out to be a problem of considerable magnitude. Various oxidants (KMnO₄, NaIO₄/RuO₂, PDC/DMF, etc.) appeared to rapidly destroy this sensitive molecule. Reasoning that oxidation to an aldehyde might allow subsequent conversion to an acid, perhaps through a Wuts-type reaction, the molecule was subjected to Swern oxidation (Scheme 20).

![Chemical Structure]

**Scheme 20**

This reaction proved to be extremely capricious, providing variable yields of the intermediate aldehyde 40 as its hydrate, grossly contaminated with unidentifiable byproducts. Purification by column chromatography provided a cleaner sample of 40, but in low yields. These discouraging results induced us to seek a different method to obtain PCA.

The Hoffman-like rearrangement of ureas is termed the Shestakov reaction. This transformation has been used to synthesize various hydrazines, including α-hydrazino acids. However, the Shestakov reaction of hydantoic acids is usually quite poor (20-30% yield). Recently, Viret reported that the use of KOCl instead of the traditional NaOCl

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34Shestakov, P. *Z. Angew. Chem.* 1903, 16, 1061.
improves the yield of this reaction to 70% or better.\textsuperscript{37} We readily confirmed such claims using L-leucine as the substrate. Moreover, we verified that leucinol, prepared by LAH reduction of the amino acid, was readily converted by aqueous sodium cyanate into urea 41, and that the latter was cleanly oxidized back to the hydantoic acid by alkaline KMnO\textsubscript{4} (Scheme 21).

\begin{center}
\includegraphics[width=\textwidth]{Scheme21.png}
\end{center}

Scheme 21

Encouraged by these results, we proceeded to treat amino alcohol 29 with aqueous NaOCN, whereupon urea 44 was obtained in 76.9 % yield. By analogy with the leucine case, we expected that the urea subunit would function as an effective N-protecting group during KMnO\textsubscript{4} oxidation of 44 to 45. Unfortunately, oxidation of 44 produced no identifiable product (Scheme 22).

\begin{center}
\includegraphics[width=\textwidth]{Scheme22.png}
\end{center}

Scheme 22

We thus examined the possibility of inducing Shestakov rearrangement starting from a hydantoic acid methyl ester. It was not possible to remove the BOC group from compound 35 without causing harm to the sensitive acetals. Fortunately, we found that conversion of TROC protected amino alcohol 32 to acid 46 occurred smoothly when RuO₄ was used as the oxidant (50 % yield, isolated as the methyl ester) (Scheme 23).

Scheme 23
IV. Conclusions

The TROC group should be easily cleaved with Zn under neutral condition, thus allowing the survival of the acetals. The experiments discussed herein clearly point the way to the completion of the synthesis of PCA. Amino ester 46 would simply be treated under Shestakov conditions to furnish the corresponding hydrazine. Cyclization as described earlier by Hughes and Clardy would produce MOM-protected PCA 49 (Scheme 24). Inclusion of the latter into a peptide would then become possible using well-established methods of protein synthesis.

![Scheme 24](image-url)
IV. EXPERIMENTAL

General Experimental Protocols.

Melting points (mp) were determined on a Fisher-Johns hot stage melting point apparatus, and are uncorrected. Infrared (IR) spectra were recorded on a Nicolet 205 FT-IR Spectrometer and are reported in wavenumbers (cm$^{-1}$). $^1$H NMR (250 MHz) and $^{13}$C NMR (62.5 MHz) spectra were measured in CDCl$_3$ solutions and were determined on a Bruker AC-250 instrument unless otherwise noted. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane ($\delta = 0$) as the internal standard, and coupling constants are in hertz. The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, cm = complex multiplet. Mass spectra (MS) were obtained on a Finnigan 3300 quadrupole mass spectrometer at 70 eV, unless otherwise noted, using direct probe insertion at temperatures of 150-300$^\circ$ C. High resolution mass spectra (HRMS) were obtained under similar conditions using a CEC 21-110B instrument.

Analytical thin layer chromatography (TLC) and preparative TLC were performed on Merck precoated analytical plates, 0.25 mm thick, silica gel 60 F$_{254}$. Column chromatography was performed on grade 62 silica gel, 60-200 mesh. Reagents and solvents were commercial grades and were used as supplied with the following exceptions:

- Dichloromethane: distilled over calcium hydride
- Tetrahydrofuran: distilled from sodium benzophenone ketyl
- Ethyl acetate: distilled at atmospheric pressure
- Methanol: dried over 4 Angstrom molecular sieves

All moisture or oxygen sensitive reactions were conducted under an argon atmosphere.
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Alkylation of acetyl enamide with methyl vinyl ketone, compound 22

To a solution of the acetyl enamide (0.295 g, 1.50 mmol) in CH₂Cl₂ (7.5 mL) was added methyl vinyl ketone (0.136 g, 1.95 mmol) via a syringe. After cooling down to −20° C (ethylene glycol/dry ice), BF₃OEt₂ (0.276 g, 1.95 mmol) was added slowly via a syringe. The reaction mixture was stirred for 4 h. TLC showed the reaction was complete (70 % EtOAc/hexane). After addition of aq. Na₂CO₃, the reaction mixture was extracted with CH₂Cl₂. The crude compound was purified by silica gel chromatography (30 % EtOAc/hexane).

Wt. of purified compound 0.0801g (20%) yellow oil.
Alkylate of acetyl enamide

$^1$H NMR: 6.36 ($^1$H, s), 4.91 ($^1$H, dt; $J=9.8$, 9.8, 6.1 Hz), 4.53 ($^1$H, dd; $J=8.6$, 8.6 Hz), 4.18 ($^1$H, dd; $J=8.7$, 8.7 Hz), 3.90 ($^1$H, dt; $J=9.8$, 8.5, 8.5 Hz), 2.57 ($^2$H, dd; $J=8.4$, 7.0 Hz), 2.60-2.04 (4H, m), 2.15 (3H, s), 2.11 (3H, s).

$^{13}$C NMR: 207.3, 170.1, 153.0, 117.5, 116.5, 70.0, 67.2, 54.6, 41.4, 32.2, 29.9, 27.3, 20.8.

IR: 3422.3, 2977.3, 2930.8, 1748.8, 1416.9, 1376.9, 1237.5, 1044.9.

Low Resolution MS: 267 ($M^+$), 210, 212, 211, 162, 149 (100 %), 133, 119, 105, 104, 82, 78, 42, 27.

High Resolution MS: Expected Mass: 267.110645
Observed Mass: 267.10921
Alkylation compound 22
Alkylation compound 22
MOM-Protected Enamide 26

Acetyl enamide 21 (5.34 g, 27.1 mmol) was added to a suspension of K$_2$CO$_3$ (3.74 g, 27.1 mmol) in MeOH (100 mL). The mixture was stirred under argon for 2 h. TLC showed completion. The reaction mixture was diluted with EtOAc (150 mL) and celite was added to the solution. The mixture was filtered through a fine porosity sintered funnel. The filtrate was concentrated in vacuo and a dark brown solid was obtained. The residue was suspended in 150 ml of CH$_2$Cl$_2$ and then filtered through a bed of celite. The filtrate was concentrated in vacuo to afford 2.99 g (81.8 %) of the hydroxy enamide as a yellow oil.

Hydroxy enamide 25

$^1$H NMR: 6.60 (1H, m), 5.01 (1H,m), 4.67 (1H, t ; J=9.0, 9.0 Hz ), 4.22 (1H,t ; J= 9.0, 9.0 Hz ), 3.90 (2H, m), 2.55 (1H, m), 2.25 (1H, m).

This sensitive compound was not thoroughly characterized. Rather, it was immediately protected as follows. To a solution of the hydroxy enamide (0.916 g, 5.91 mmol ) in CH$_2$Cl$_2$ (10 mL) was added Hunig's base (3.43 g, 26.7 mmol). MOM-Cl (1.66 g, 20.8 mmol ) was added with cooling below the surface of the reaction mixture via a syringe. The reaction mixture was then heated to 50$^\circ$C for 24 h. The mixture was poured into saturated aq. NaHCO$_3$ and extracted with CHCl$_3$. The extracts were dried (Na$_2$SO$_4$) and
Hydroxy enamide 25
concentrated in vacuo. The crude product (0.980 g) was passed through silica gel (10 g) and eluted with 50% EtOAc/hexane to afford 0.830 g (71.0 %) of 26 as a yellow oil.

**Compound (±) 26 (Equatorial Stereoisomer).**

1H NMR: 6.54 (1H, ddd; J = 7.8; 2.6; 1.5 Hz), 4.98 (1H, ddd; J = 7.8, 5.7, 2.3 Hz), 4.75 (1H, d; J = 7.0 Hz), 4.64 (1H, dd; J = 8.8; 8.5 Hz), 4.63 (1H, d; J = 7.0 Hz), 4.20 (1H, dd; J = 8.8; 8.7 Hz), 3.87 (1H, ddd; J = 9.2; 8.8; 8.7 Hz), 3.74 (1H, dt; J = 9.5; 9.5; 5.7 Hz), 3.36 (3H, s); 2.58 (1H, ddt; J = 17.4; 5.7; 5.7; 1.5 Hz), 2.17 (1H, ddt; J = 17.4; 9.4; 2.5; 2.5 Hz).

13C NMR: 154.1, 121.3, 105.5, 95.6, 74.0, 68.0, 55.7, 55.5, 29.2.

IR: 3083.6, 2917.6, 1768.7, 1649.2, 1483.2, 1423.4, 1337.1, 1264.1, 1144.5, 1044.9, 985.2, 912.1, 759.4.

Low Resolution MS: 200 (M++1), 199 (M+, 100 %), 154, 137, 136, 83, 47.

High Resolution MS: Expected Mass: 199.0844
Observed Mass: 199.0841

![Graphical representation of MS data for compound 26]
Compound (±) 26 (Equatorial Stereoisomer)
Compound (±) 26 (Equatorial Stereoisomer).
**Compound (+) 26-a (equatorial stereoisomer).**

$^1$H NMR: 6.56 (1H, ddd; J=7.8, 2.7, 1.6 Hz), 4.99 (1H, ddd; 7.8, 5.6, 2.2 Hz), 4.76 (1H, d; J=7.0 Hz), 4.65 (1H, dd; J=9.9, 8.5 Hz), 4.64 (1H, d; J=7.0 Hz), 4.21 (1H, dd; J=8.8, 8.8 Hz), 3.88 (1H, dt; J=8.8, 8.7, 8.7 Hz), 3.75 (1H, dt; J=9.5, 9.5, 5.8 Hz), 3.37 (3H, s), 2.59 (1H, ddt; J= 17.3, 5.7, 5.7, 1.5 Hz), 2.17 (1H, ddt ; J=17.3, 9.4, 2.4, 2.4 Hz).

$^{13}$C NMR: 154.1, 121.4, 105.5, 95.6, 74.0, 68.0, 55.7, 55.6, 29.3.

IR : 3435.5, 2924.2, 1762.1, 1649.2, 1416.8, 1337.1, 1270.7, 1157.8, 1118.0, 1038.3, 918.8.

Low Resolution MS: 200 (M$^+$+1), 199 (M$^+$), 154, 137, 136, 126, 107, 86, 45 (100 %), 41, 39, 29, 27.

High Resolution MS: Expected Mass: 199.084435
Observed Mass: 199.08406

Optical Rotation:  $[^\alpha]_D^{25}$ = +93.5° (c= 0.515; EtOH)
Compound (+)-26-a (equatorial stereoisomer)
Compound (+) 26-a (equatorial stereoisomer)
**Compound (-) 26-b (axial stereoisomer)**

**1H NMR:**
6.63 (1H, ddd; J = 8.0; 2.4; 1.4 Hz), 4.95 (1H, ddt; J = 6.6, 6.6, 2.3, 1.2 Hz), 4.76 (1H, d; J = 7.1 Hz), 4.64 (1H, d; J = 7.1 Hz), 4.45 (1H, t; J = 8.6 Hz), 4.36 (1H, dd; J = 8.1; 8.1 Hz), 4.06 (1H, br.d; J = 9.0 Hz), 4.00 – 3.97 (1H, m), 3.37 (3H, s), 2.47 – 2.37 (1H, m), 2.30 – 2.20 (1H, m).

**13C NMR:**
154.8, 122.2, 104.4, 95.3, 66.9, 64.2, 55.9, 55.3, 26.8.

**IR:**
3442.2, 2924.2, 1755.5, 1649.2, 1476.6, 1423.4, 1290.6, 1151.2, 1078.1, 1038.3, 985.2, 925.4.

**Low Resolution MS:**
200 (M+1), 199 (M+), 154, 137, 136, 85, 79, 56, 45 (100 %), 27.

**High Resolution MS:**
Expected Mass: 199.084435
Observed Mass: 199.08406

**Optical Rotation:**
\([\alpha]_D^{25} = -30.0^\circ\) (c=0.924; EtOH)
Compound (-) 26-b (axial stereoisomer)
Compound (-) 26-b (axial stereoisomer)
The ozonolysis of MOM-enamide, compound 28

Ozone was bubbled through a solution of the racemic enamide (1.40 g, 7.0 mmol) in CH₂Cl₂ (16 mL) and MeOH (49 mL) at −78°C till the blue color persisted (45 min), and TLC showed completion (8% MeOH/CHCl₃). The excess ozone was purged with argon gas at −78°C and Me₂S (6.1 mL, 70 mmol) was added to the mixture at 0°C. The reaction mixture was stirred at room temperature for 4 h. Then the volatiles were pumped off and to the residue was added a solution of CeCl₃·7H₂O (2.75 g, 7.4 mmol) in MeOH (18 mL) followed by HC(OMe)₃ (7.7 mL, 70 mmol).

The mixture was stirred for 12 h and then poured into satd. aq. NaHCO₃ and extracted with CHCl₃ followed by EtOAc. The combined organic extracts were passed through Na₂SO₄ and concentrated in vacuo to afford 1.80 g of crude acetal oxazolone as a brown oil. The compound was purified on a column of silica gel (30 g) and eluted with 50% EtOAc/hexane (small amount of Et₃N added into the solvent for keeping away from the decompostition of the compound). Purification afforded 1.05 g (60.0 %) of racemic acetal oxazolone 28 as a yellow oil.
Acetal oxazolone (±)28

$^1$H NMR: 6.28 (1H, br.d; $J=8.0$ Hz), 4.66 (1H, d; $J=7.0$ Hz), 4.60 (1H, d; $J=7.0$ Hz), 4.50 (1H, t; $J=5.4$, 5.4 Hz), 4.40 (1H, dd; $J=8.9$, 8.9Hz), 4.25 (1H, dd; $J=8.8$, 5.3 Hz), 3.90 (1H, dt; $J=8.8$, 5.1, 4.7Hz), 3.68 (1H, dt; $J=5.4$, 5.4, 5.4 Hz), 3.36 (3H,s), 3.30 (3H,s), 3.28 (3H,s), 1.78 (2H, dd; $J=5.5$, 5.5 Hz).

$^{13}$C NMR: 159.8, 101.3, 96.6, 76.0, 66.8, 55.9, 55.1, 53.4, 52.8, 34.1.

IR: 3308.6, 2934.0, 2833.1, 1759.7, 1406.6, 1226.5, 1111.2, 1024.8, 923.9.

Low Resolution MS: 186, 156, 131, 100, 83 (100%), 75, 59, 48, 45, 35.

High Resolution MS: Expected Mass: 249.121205
Observed Mass: 249.12028
Acetal oxazolone (±)28
Acetal oxazolone (±)28
**Acetal oxazolone (−) 28-a (anti stereoisomer)**

**1H NMR:**
5.76 (1H, br.s), 4.69 (1H, d; J=7.0 Hz), 4.62 (1H,d; J=7.0 Hz),
4.53 (1H, t; J=5.3, 5.3 Hz), 4.45 (1H,dd; J=8.8, 8.8 Hz), 4.27
(1H, dd; J=8.8, 5.3 Hz), 3.92 (1H, dt; J=8.8, 4.5, 1.0 Hz), 3.71
(1H,dt; J=5.4, 5.4, 5.4 Hz), 3.38 (3H, s), 3.34 (3H, s), 3.32 (3H,
s), 1.842 (1H, dd; J=5.5, 5.5 Hz), 1.838 (1H, dd; J=5.2, 5.2 Hz).

**13C NMR:**
159.4, 101.3, 96.5, 76.2, 67.2, 56.0, 55.0, 53.7, 52.7, 34.1.

**IR :**
3296.1, 2930.8, 2831.2, 1755.5, 1403.5, 1230.8, 1157.8,
1104.7, 1058.2, 1031.6, 925.4.

**Low resolution MS:**
217, 186, 131, 124, 87 (100 %), 75, 71, 59, 45, 29.

**High Resolution MS:**
Expected Mass: 249.121205
Observed Mass: 249.12028

**Optical Rotation:**
\([\alpha]_D^{25} = -5.9^\circ\) (c=0.640; EtOH).
Acetal oxazolone (−) 28-a (anti stereoisomer)
Acetal oxazolone (−) 28-a (anti stereoisomer)
Acetal oxazolone (-) 28-a (anti stereoisomer)
Acetal Oxazolone (+) 28-h (syn stereoisomer)

$^1$H NMR: 6.47 (1H, br.s), 4.68 (1H, d; J=7.1 Hz), 4.64 (1H, d; J=7.1 Hz), 4.50 (1H, t; J=5.4, 5.4 Hz), 4.39 (1H, dd; J=8.8, 8.8 Hz), 4.12 (1H, dd; J=8.8, 5.9 Hz), 3.90 (1H, dt; J=8.6, 6.2, 6.2 Hz), 3.59 (1H, dt; J=6.0, 6.0, 6.0 Hz), 3.37 (3H, s), 3.30 (6H, s), 1.74 (2H, t; J=5.6, 5.6 Hz).

$^{13}$C NMR: 159.5, 101.4, 97.2, 78.2, 66.6, 55.9, 55.6, 53.3, 34.3.

IR: 3296.1, 2930.8, 2831.2, 1755.5, 1403.5, 1230.5, 1157.8, 1104.7, 1058.2, 1031.6, 925.4.

Low resolution MS: 218, 202, 186, 159, 156, 131, 100, 87, 75, 59, 45, 30, 29 (100 %), 28.

High Resolution MS: Expected Mass: 249.121205
Observed Mass: 249.12028

Optical Rotation: $[\alpha]_D^{25} = +31.4^\circ$ (c=0.236; EtOH)
Acetal oxazolone (+) 28-h (syn stereoisomer)
Acetal oxazolone (+) 28-h (syn stereoisomer)
Acetal oxazolone (+) 28-b (syn stereoisomer)
**Preparation of amino alcohol 29**

To a solution of oxazolone (0.113 g, 0.452 mmol) in EtOH (5 mL) was 20% aq. NaOH (0.226 mL, 1.13 mmol). The solution was refluxed under Ar for 18 h. TLC showed completion (8% MeOH/CHCl₃). The pH of the solution was adjusted to 7 with aq. NH₄Cl and then the solution was freeze dried. A white solid was obtained. This residue was thoroughly washed with acetone and insoluble solids were filtered off. The solvent was concentrated in vacuo and a brown oil was obtained.

Wt. of brown oil 0.102 g (100 %).
Amino alcohol 29

**1H NMR:** 4.74 (1H, d; J=6.7 Hz), 4.68 (1H, d; J=6.7 Hz), 4.56 (1H, dd; J=5.2, 5.2 Hz), 4.01 (1H, br.s), 3.90 (1H, br.d; J=8.0 Hz), 3.80-3.74 (1H, br), 3.56 (1H, br.s), 3.40 (3H, s), 3.33 (6H, s), 1.91 (2H, br.s).

**13C NMR:** 101.5, 97.3, 74.1, 58.4, 56.1, 55.8, 53.2, 34.5.

**IR:** 2939.3, 2843.0, 1608.2, 1517.6, 1460.9, 1443.9, 1393.0, 1200.4, 1132.4, 1041.8, 917.2.

**Low Resolution MS:** 213, 207, 202, 98, 91, 86, 84 (100%), 60, 51, 49, 42, 29.

**High Resolution MS:**
- **Expected Mass:** 223.141945
- **Observed Mass:** 223.14195

![Graph of TSH9](Image)
Amino alcohol 29

TSH10 AMINO ACID
Amino alcohol 29
Preparation of N-protected amino alcohol with CBZ-Cl 30

Amino alcohol 29 (0.0985 g, 0.442 mmol) was dissolved in sat. aq. NaHCO₃ (1 mL). Dioxane (2 mL) was added, and the solution was placed under inert atmosphere. Carbobenzyloxy chloride (0.0904 g, 0.530 mmol) was added via a syringe. The reaction mixture was stirred for 2 h, after which time TLC showed completion (8% MeOH/CHCl₃). More aq. NaHCO₃ solution was added, and the reaction mixture was filtered through a celite bed. The aq. phase was extracted with EtOAc and the combined organic phases were concentrated in vacuo to afford 0.138 g (87.2%) of CBZ amino alcohol 30 as a yellow oil.
**Chz group protected amino alcohol 30**

**1H NMR:**
7.36-7.29 (5H, m), 5.77 (1H, br.d; J=7.9 Hz), 5.10 (2H, s), 4.64 (2H, s), 4.50 (1H, dd; J=5.7, 5.7 Hz), 3.91 (1H, dd; J=11.8, 4.0 Hz), 3.81 (1H, m), 3.69 (2H, m), 3.39 (3H, s), 3.31 (3H, s), 3.30 (3H, s), 1.89 (2H, dd; J=6.2,5.6 Hz).

**13C NMR:**
156.6, 136.4, 128.5, 128.1, 128.0, 101.7, 97.7, 77.8, 66.8, 62.0, 56.1, 55.2, 53.0, 52.7, 35.8.

**IR :**
3322.6, 2944.1, 2831.2, 1735.5, 1702.3, 1695.7, 1536.3, 1450.0, 1390.2, 1337.1, 1250.8, 1137.9, 1025.0, 912.1.

**Low Resolution MS:**
294, 250, 239, 232, 208, 194, 150, 148, 104, 91 (100%), 75, 45, 31.

**High Resolution MS:**
- Expected Mass: 357.178715
- Observed Mass: 357.17863
CBZ amino alcohol 30
CBZ amino alcohol

Subtraction Result 28 Jul 91 13:25:25
CBZ amino alcohol 30

7/14 COZAMINO ALCOHOL
= 35

AMP.: 00131000

>350 x 10

15
The procedure of N-protection & Hydrolysis of acetal oxazolone

Method A : Hydrolysis then N-protection

To a solution of the racemic oxazolone (0.514 g, 2.06 mmol) in EtOH (25 mL) was added aq. NaOH (20 % solution, 2.5 eq). The solution was refluxed under argon for 18 h and TLC showed completion (8% MeOH/CHCl₃). The alcohol was pumped off and the pH of the residue was adjusted to 8 using dry ice as the source of CO₂. Then, THF (10 mL) and 10% aq NaOH solution (4 mL) were added to the solution, followed by di-tert-butyl-dicarbonate (0.560 g, 2.58 mmol). The reaction mixture was stirred at room temperature for 6 h. TLC showed the reaction was finished (100 % EtOAc). The reaction mixture was extracted with EtOAc. The combined extracts were dried and concentrated in vacuo to afford 0.391 g (58.8 % from acetal oxazolone) of BOC amino alcohol 31 as a brown oil.

Method B : N-protection then hydrolysis

The acetal oxazolone (the equatorial compound) (0.173 g, 0.696 mmol) was dissolved in THF. (BOC)₂O (0.197g, 0.905 mmol), Et₃N (0.116 mL, 0.835 mmol) and DMAP (0.0170g, 0.129 mmol) were added into the solution. The reaction mixture was stirred at room temperature for 4 h. TLC showed the reaction was complete (100 % EtOAc). The
reaction mixture was quenched by sat. NaHCO₃ and extracted with EtOAc.

Wt. of concentrated residue 0.240 g as a brown oil.

BOC acetal oxazolone (0.234 g, 0.670 mmol) was dissolved in MeOH (7 mL). K₂CO₃ (0.139 g, 1.01 mmol) was added into the solution and stirred at room temperature for 4 h. TLC showed the reaction was complete (100% EtOAc). K₂CO₃ was filtered off and washed with EtOAc. The solvent was evaporated and the residue was dissolved in CH₂Cl₂ and filtered off the solid with a celite bed. Concentration in vacuo afforded 0.207 g (92.1% from acetal oxazolone) of BOC amino alcohol 31 as a brown oil.

N protected amino alcohol (-)-31-a (anti stereoisomer)

**1H NMR:**

<table>
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<tr>
<th>Chemical Shift</th>
<th>Multiplicity</th>
<th>J (Hz)</th>
</tr>
</thead>
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<tr>
<td>5.45</td>
<td>1H, br.d; J=7.4 Hz</td>
<td>4.64</td>
</tr>
</tbody>
</table>

**13C NMR:**

156.5, 101.8, 97.7, 79.5, 62.4, 56.0, 54.9, 53.0, 52.7, 35.8, 28.3.

**IR:**

3455.5, 3362.5, 2924.2, 2831.2, 1722.3, 1695.7, 1516.4, 1463.3, 1376.9, 1257.4, 1171.1, 1124.6, 1038.3, 925.4.

**Low resolution MS:** 292, 260, 204, 160, 142, 128, 103, 98, 87, 86, 84, 75, 71, 67, 60, 59, 57 (100%), 45, 41, 29.

**High Resolution MS:**

Expected Mass: 323.194365

Observed Mass: 323.19302

**Optical Rotation:** 

\[ [\alpha]_D^{25} = -9.7^\circ \text{ (c=0.452; EtOH)} \]
BOC amino alcohol (−)-31a (anti stereoisomer)
ROC amino alcohol (−) 31-a (anti stereoisomer)
BOC amino alcohol (−) 31-a (anti stereoisomer)
N-protected amino alcohol (-) 31-b (syn stereoisomer)

$^1$H NMR: 4.96 (1H, br.d; J=8.5 Hz), 4.67 (1H, d; J=6.6 Hz), 4.61 (1H,d; J=6.5 Hz), 4.50 (1H, t; J=5.9, 5.9 Hz), 3.91 (1H, dt; J=6.1, 6.1, 1.7 Hz), 3.71 (1H, br), 3.66 (1H, br.d; J=4.7 Hz), 3.60 (1H, br.dd; J=8.3, 3.8 Hz), 3.39 (3H,s), 3.32 (3H, s), 3.31 (3H, s), 1.85 (2H, ddd; J=6.2, 6.2, 2.9 Hz).

$^{13}$NMR: 156.2, 101.8, 97.0, 79.6, 74.6, 63.1, 56.0, 54.5, 53.0, 35.1, 28.3.

IR: 3455.5, 2924.2, 2831.2, 1722.3, 1695.7, 1509.8, 1463.3, 1376.9, 1257.4, 1171.1, 1124.6, 1038.3, 925.4.


High Resolution MS: Expected Mass: 323.194365
Observed Mass: 323.19302

Optical Rotation: $[\alpha]_D^{25} = -60.1^\circ$ (0.818; EtOH)
BOC amino alcohol (−) 31-b (syn stereoisomer)
BOC amino alcohol (−) 31-b (syn stereoisomer)
BOC amino alcohol (-) 31-b (syn stereoisomer)
Preparation of N-protected amino alcohol with TROC group 32

Amino alcohol 29 (0.0451 g, 0.202 mmol) was dissolved in aq. NaHCO$_3$ (1 mL). Dioxane (2 mL) was added and the solution was placed under inert atmosphere. 2,2,2-Trichloroethyl chloroformate (0.0514 g, 0.242 mmol) was added via a syringe and the reaction mixture was stirred for 30 min. TLC showed completion (12 % MeOH/CHCl$_3$). More aq. NaHCO$_3$ was added and the reaction mixture was filtered through a celite bed. The aq. phase was extracted with EtOAc. The combined extracts were concentrated in vacuo to afford 0.0705 g (87.6 %) of 32 as a colorless oil.
Troc group protecting amino alcohol 32

$^1$H NMR: 6.07 (1H, br.d; J=7.8 Hz), 4.76 (1H, d; J=11.9 Hz), 4.69 (1H,d; J=11.9 Hz), 4.66 (2H, s), 4.51 (1H, t; J=5.8, 5.8 Hz), 3.96 (1H, dd; J=12.8, 4.6 Hz), 3.84 (1H, dd; J=11.1, 6.1 Hz), 3.70 (2H, m), 3.42 (3H, s), 3.32 (3H, s), 3.31 (3H, s), 1.91 (2H, dd; J=5.7, 5.7 Hz).

$^{13}$C NMR: 154.7, 101.7, 97.8, 95.5, 77.8, 74.5, 61.7, 56.1, 55.3, 53.1, 52.8, 35.8.

IR: 2935.7, 2840.8, 1738.7, 1717.6, 1548.8, 1522.5, 1464.5, 1390.6, 1232.4, 1153.3, 1126.9, 1100.6, 1032.0.


High Resolution MS: Expected Mass: 397.046140
Observed Mass: 397.04567
TROC amino alcohol 32
TROG amino alcohol 32
TROC amino alcohol 32
**KMnO₄ oxidation of BOC protecting alcohol, compound 34**

To a slurry of the alcohol (the equatorial compound) (0.0937g, 0.290 mmol) in water was added aq. NaOH (1N, 0.8 eq), followed by KMnO₄ (0.148 g, 3 eq). The mixture was stirred at room temperature for 24 h. TLC showed that slight amount of the alcohol remained (100 % EtOAc). To the reaction mixture was added solid NaHSO₃ till the brown ppt was consumed and the reaction mixture became clear. Water (10 mL) was added into the reaction and the pH was adjusted to 9. The aqueous phase was extracted with EtOAc, then, acidified to pH 3.5-4 with 1N HCl and extracted again with EtOAc. The combined extracts were concentrated in vacuo to afford 0.0542 g (65.1 %) of the acid 34 as a colorless oil.

**Methylation of BOC acids, compound 35**

The acid (0.0542 g) was dissolved in ether. A solution of CH₂N₂ in ether was added to the acid solution at 0° C. TLC showed the methylation to be complete (100 % EtOAc). CH₂N₂ was purged with argon for 10 min. Then, the reaction mixture was passed through a Na₂SO₄ column and the recovered solution was concentrated in vacuo. The ester was purified by column chromatography on silica gel (1 g; eluted with 50% EtOAc/hexane) to afford 0.0513 g (50.4 % from alcohol) of methyl ester 35 as a colorless oil.
N-protected amino ester (−) 35-a (anti stereoisomer)

1H NMR: 5.73 (1H, br.d; J=8.9 Hz), 4.69 (1H, d; J=7.0 Hz), 4.62 (1H, d; J=7.0 Hz), 4.53 (1H, dd; J=7.5, 4.2 Hz), 4.43 (1H, dd; J=8.8, 2.6 Hz), 3.93 (1H, ddd; J=4.5, 4.4, 3.0 Hz), 3.76 (3H, s), 3.41 (3H, s), 3.30 (3H, s), 1.92 (2H, m).

13C NMR: 170.5, 155.5, 101.5, 97.1, 79.8, 76.8, 56.7, 56.0, 53.1, 52.5, 52.2, 35.3, 28.3.

IR: 3362.5, 2924.2, 2831.2, 1755.5, 1722.3, 1509.8, 1443.4, 1370.3, 1157.8, 1124.6, 1104.7, 1038.3, 925.4.

Low Resolution MS: 288, 246, 233, 204, 188, 177, 133, 126, 118, 116, 101, 87, 75 (100 %), 59, 57, 45, 41, 31, 29.

High Resolution MS: Expected Mass: 351.189275
Observed Mass: 351.18919

Optical Rotation: [α]D²⁵= −22.3° (c=0.520; EtOH)
BOC amino ester (-35-a (anti stereoisomer))
BOC amino ester (−)-35-a (anti stereoisomer)
BOC amino ester (−) 35-a (anti stereoisomer)
**N-protected amino ester (+) 35-b** (syn stereoisomer)

**1H NMR:**
5.27 (1H, br.d; J=9.7 Hz), 4.59 (1H, d; J=6.9 Hz), 4.50 (1H,d; J=6.9 Hz), 4.49 (1H, t; J=5.6, 5.6 Hz), 4.35 (1H, dd; J=9.8, 1.9 Hz), 4.21 (1H, dt; J=6.8, 6.8, 1.8 Hz), 3.74 (3H, s), 3.32 (6H, s), 3.28 (3H,s), 1.88 (2H, m), 1.44 (9H, s).

**13C NMR:**
171.4, 156.0, 101.6, 96.5, 80.0, 75.3, 56.8, 56.0, 53.2, 52.8, 52.3, 35.1, 28.2.

**IR:**
3362.5, 2937.5, 2831.2, 1755.5, 1722.3, 1509.8, 1436.7, 1376.9, 1257.4, 1217.6, 1157.8, 1124.6, 1104.7, 1031.6, 918.8.

**Low Resolution MS:**
260, 233, 218, 204, 188, 184, 177, 160, 156, 133, 126, 118, 116, 101, 88, 75 (100 %), 59, 57, 45, 41, 29.

**High Resolution MS:** Expected Mass: 351.189275
Observed Mass: 351.18919

**Optical Rotation:**
[α]D²⁵+=20.7° (c=0.492; EtOH)
BOC amino ester (+) 35-b (syn stereoisomer)
BOC amino ester (+) 35-b (syn stereoisomer)
BOC amino ester (+) 35-b (syn stereoisomer)
Preparation of Ph₂P(O)ONH₂

In a 500 ml 3-necked flask fitted with a mechanical stirrer and a dropping funnel was placed aqueous NH₂OH·HCl (13.5 mL of 6.6 M solution, 88.6 mmol) and a trace of phenolphthalein. An aqueous NaOH solution (10.6 mL of 7.1 M solution, 75.2 mmol) was added with high speed stirring at 10–15°C, followed by dioxane (43 mL). The flask was cooled to 0°C and the mechanical stirrer was set to full speed. A solution of diphenylphosphinic chloride (7.64 g, 32.2 mmol) in dioxane (18.5 mL) was added through the dropping funnel rapidly in a single portion. A white solid started forming after one min. The mixture was stirred for 30 min at high speed. The contents of the flask was poured in ice cold water (600 mL). The solid was filtered off, washed with ice cold water till washings were neutral to pH paper. The wet solid was dried over P₂O₅ in a vacuum desiccator for 48 h.

Wt. of dry solid 4.84g (64.5 %), melting point 134-138°C.
Preparation of amino oxazolone 36

In a flame dried 100 ml, 3-necked flask was placed NaH (0.0501 g, 1.04 mmol) under argon and washed with hexane. A solution of the oxazolone (0.200 g, 0.803 mmol) in DMF (10 mL) was added to at 0°C. After the evolution of H₂ ceased, Ph₂P(O)ONH₂ (0.243 g, 1.04 mmol) was added to the reaction mixture in a single portion. The brown coloured reaction mixture was stirred at 25°C for 3 h, at the end of which TLC showed virtual completion (8 % MeOH/CHCl₃). The reaction mixture was poured in water and extracted with CHCl₃. The organic extract was dried (Na₂SO₄) and concentrated in vacuo. The residue was heated at 50°C/0.1mm for 8 h to remove DMF. It afforded 0.198 g (93.3 %) of amino oxazolone 36 as a brown oil.

IR : 3352.3, 3215.2, 2930.5, 2830.3, 1759.8, 1638.5, 1480.3, 1427.5, 1374.8, 1216.6, 1153.3, 1121.7, 1100.6, 1032.0, 974.0, 921.3.
Amino oxazolone 36
Cyclization of the amino oxazolone 37

Method A (catalyst; quinolinium camphorsulfonate (QCS))

A solution of crude amino oxazolone (0.283 g, 1.07 mmol) and QCS (0.0386 g, 0.107 mmol) in acetonitrile (6 mL) was refluxed under argon for 3 h. TLC showed complete cyclization (EtOAc, double development). The reaction mixture was passed through a short plug of silica gel (0.5 g) to remove QCS. The solvent was pumped off and the residue was applied to 6 g of silica gel. Elution with 50% EtOAc in hexane containing a small amount of Et₃N (added to prevent decomposition of the acetal group). The combined eluates were concentrated in vacuo to afford 0.112 g (38.5%) of tetrahydropyridazine 37 as a yellow oil.

Method B (catalyst; small amount of 1N HCl solution)

To a solution of crude amino oxazolone (0.121 g, 0.464 mmol) was added 5 drops of 1N HCl. The reaction mixture was stirred at room temperature for 1 h. TLC showed complete cyclization (8% MeOH/CHCl₃). Saturated aq. Na₂CO₃ was added to the reaction, and the mixture was passed through a short plug of Na₂SO₄. Evaporation gave a crude compound, which was purified by column chromatography over 1.6 g of silica gel (eluted with 50/50 EtOH/hexane) to afford 0.0678 g (73.1%) of pure compound 37.
Tetrahydropyridazine 37

1H NMR: 7.00 (1H, dd; J=3.8, 1.6 Hz), 4.72 (1H, d; J=7.1 Hz), 4.70 (1H, dd; J=7.7, 1.5 Hz), 4.64 (1H, d; J=7.1 Hz), 4.21 (1H, dd; J=9.2, 9.2 Hz), 3.83-3.77 (2H, m), 3.36 (3H, s), 2.84-2.72 (1H, m), 2.34-2.22 (1H, m).

13C NMR: 153.2, 144.8, 96.0, 70.8, 67.4, 55.8, 54.9, 31.8.

IR: 2937.5, 1788.7, 1622.6, 1476.6, 1383.6, 1343.7, 1277.3, 1217.6, 1144.5, 1091.4, 991.8, 918.8.

Low Resolution MS: 200 (M+), 167, 155, 149, 138, 130, 111, 100, 86, 84 (100 %), 83, 51, 49, 30, 27.

High Resolution MS: Expected Mass: 200.079680
Observed Mass: 200.07950

910714
TSHB PURE PYPIDADINE
AMP.: 0002195E
Tetrahydropridazine 37
Tetrahydroxazine 37
Hydrolysis of the oxazolone, compound 38

A solution of the oxazolone (0.0678 g, 0.339 mmol) in 0.1N NaOH was left standing at room temperature for 90 min, after which time TLC showed complete hydrolysis (8 % MeOH/CHCl₃). The aq. mixture was stirred with Amberlite IRC-50 resin (0.1 g) for 15 min. The pH of the solution was checked using pH paper and adjusted to 7. The resin was filtered off, washed with water, and the combined aq. phases were freeze dried. The yellow solid thus obtained, was washed with acetone, and the combined organic extracts were passed through a short plug of Na₂SO₄. Concentration in vacuo afforded 0.0583 g (98.8 %) of alcohol 38 as a yellow oil.
Hydrazono alcohol 38

$^1$H NMR: 6.70 (1H, dd; J=2.7, 2.7 Hz), 5.86 (1H, br.s), 4.70 (1H, d; J=6.8 Hz), 4.65 (1H, d; J=6.8 Hz), 3.88 (1H, dd; J=7.6, 1.7 Hz), 3.82 (1H, dd; J=6.4, 2.5 Hz), 3.67 (1H, dd; J=11.3, 6.5 Hz), 3.36 (3H, s), 2.98 (1H, ddd; J= 5.9, 5.9, 3.1 Hz), 2.60 (1H, ddd; J=18.6, 6.7, 3.0 Hz), 2.21 (1H, ddd; J=18.6, 7.9, 1.7 Hz).

$^{13}$C NMR: 139.3, 95.4, 68.7, 61.3, 57.6, 55.6, 31.8.

IR: 3352.7, 2939.3, 2893.9, 2843.0, 1630.8, 1613.9, 1449.6, 1387.3, 1347.6, 1313.7, 1217.4, 1149.4, 1104.1, 1041.8, 911.5.

Low Resolution MS: 174 (M+), 143, 113, 101, 95, 85, 83, 81, 56, 45 (100 %), 31, 29.

High Resolution MS: Expected Mass: 174.100420
Observed Mass: 174.10071

910763
n 54

TSH11

AMP: 00054208
Hydrazono alcohol 38
Hydrazono alcohol 38
Selective N-protection of hydrazono alcohol 39

To a stirred suspension of the hydrazono alcohol (0.0508 g, 0.322 mmol) in acetonitrile (2.5 mL) was added the octanone ester (0.0938 g, 0.354 mmol) and HOBt (0.039 g, 0.289 mmol). The mixture was stirred under Ar for 2 days, after which the reaction was complete (TLC; 8% MeOH/CHCl₃). The solvent was pumped off and the residue was purified on silica gel (1 g). Elution with 10% EtOAc/hexane provided fractions containing the starting octanone ester. Further elution with 90% EtOAc/hexane furnished 0.0554 g (60.6%) of 39. This material was contained with HOBt, which could not be removed.
N-protected hydrazono alcohol 39

**1H NMR:** 7.90-7.27 (due to HOBT), 7.25 (1H, not clear due to CHCl₃ peak), 4.77 (1H, d; J=6.8 Hz), 4.74 (1H, d; J=7.0 Hz), 4.70-4.30 (huge broad peak due to H₂O of HOBT), 4.06 (1H, dd; J=12.1, 3.8 Hz), 3.92 (1H, dd; J=12.1, 2.6 Hz), 3.39 (3H, s), 2.85 (1H, m), 2.55 (1H, ddd; J=16.3, 16.3, 8.1 Hz), 2.26 (1H, ddd; J=14.0, 10.0, 6.5 Hz), 1.60-1.47 (2H, br.m), 1.30-1.16 (10H, br.m), 0.84 (3H, t; J=6.70, 6.70 Hz).

**IR:** 3316.0, 2937.5, 2851.2, 1755.5, 1735.5, 1702.3, 1669.1, 1635.9, 1463.3, 1390.2, 1303.9, 1217.6, 1157.8, 1104.7, 1044.9, 912.1.

**Low Resolution MS:** 301 (M⁺+1), 300 (M⁺), 274, 272, 256, 174, 143, 135, 127, 119, 107, 91, 90, 81, 45, 29, 28 (100 %), 27.

**High Resolution MS:** Expected Mass: 300.204880
Observed Mass: 300.20451
N-protected hydrazono alcohol 39
N-protected hydrazono alcohol 39
The formation of urea L-leucinol from L-leucine, compound 41

LAH (1.45 g, 38.2 mmol) was placed into a 100 ml 3 neck flask (flame-dried) under Ar. Ether (30 mL) was added into the flask and the solution was cooled to -10°C (MeOH/dry ice). L-leucine (1 g, 7.63 mmol) was added via a spatula and 2 h later the reaction mixture was warmed up to 0°C (ice/water). After another 2 h, the reaction mixture was heated to reflux for 3 h. Water (10 mL) was cautiously added into the reaction mixture (white precipitate appeared). The combined aq. phases were separated and evaporated. The white solid residue was washed with MeOH. The combined washes were filtered off and evaporated to afford 2.33 g of crude L-leucinol.

To a solution of crude L-leucinol (1.17g) in water (5 mL) was added KOCN (1.56 g, 19.2 mmol). The reaction mixture was stirred at 60°C overnight, after which time TLC showed completion (50 % MeOH/CHCl₃). The reaction mixture was cooled to room temperature and some white precipitate appeared. The solution was filtered, cooled to 0°C and acidified with conc. HCl. After quenching the acid with 10 % aq. NaOH, the solvent was pumped off and a white solid was obtained. The solid was suspended in MeOH and insoluble matter was filtered off. Evaporation of the solvent afforded 0.542 g (88.3 %) of
the urea alcohol as white crystals, m.p. 94–96° C.

**L-leucinol urea**

\(^1\)H NMR(DMSO-d6): 5.76 (1H, br.d; J=14.6 Hz), 5.46 (1H, br.s), 5.39 (1H, br.s), 3.39 (1H, dd; J=5.6, 3.8 Hz), 3.19 (1H, dd; J=10.1, 5.0 Hz), 1.70-1.50 (1H, m), 1.32-1.13 (2H, m), 0.86 (6H, t; J=7.2, 7.2 Hz).

\(^13\)C NMR(DMSO-d6): 158.7, 64.6, 48.9, 40.8, 24.3, 23.5, 22.1.

IR: 3335.9, 2950.0, 2864.4, 1755.5, 1695.7, 1655.9, 1616.0, 1556.2, 1443.4, 1357.0, 1164.4, 1025.0.

Low Resolution MS: 130, 129, 103, 86, 60, 44, 31, 30 (100 %), 29, 28 (100 %), 27.

High Resolution MS: Expected Mass: 160.121160
Observed Mass: 160.12075

Optical Rotation: \([\alpha]_D^{25} = -23.0^\circ \) (c=0.250; EtOH)
L-Leucinol Urea 41

\[
\text{\begin{center}
\begin{tikzpicture}
    \node at (0,0) {\includegraphics[width=0.2\textwidth]{structure.png}};
\end{tikzpicture}
\end{center}
}\]

\[\text{NHCONH}_2\]
L-Leucinol Urea 41
Oxidation of L-leucinol urea

To a slurry of L-leucinol urea (0.207 g, 1.29 mmol) in water (8 mL) was added 1N NaOH (1.29 mL, 1.29 mmol), followed by KMnO₄ (0.624 g, 3.87 mmol). The mixture was stirred at room temperature overnight. TLC showed the reaction was complete. Solid NaHSO₃ was added till the brown ppt was consumed. More water (10mL) was added, and pH was adjusted to 4 using 1N HCl. The aqueous phase was extracted with EtOAc, and the extracts were discarded. The aqueous residue was evaporated to give a white solid. This residue consisted of a mixture of hydantoic acid and NaCl. The residue was suspended in MeOH and insoluble matter was filtered off. Evaporation of the organic phase afforded 0.0863 g (38.4 %) of urea acid, 42 as white crystals.
The Shestakov reaction.

Hydantoic acid 42 (0.498 g, 2.86 mmol) was dissolved at 0° C in 5N KOH solution (1.7 mL) and KOCl solution (1.03 M, 4.2 mL) was added. After overnight stirring at r.t. in the dark, the excess reagent was destroyed by addition of solid Na₂SO₃. A small amount of ether was added and the solution was brought to pH 1-2 by slow addition of concentrated HCl (gas evolution). The solution was extracted twice with ether and the aqueous phase was percolated through a Dowex 1×8 column, which had previously been washed with 10% aq. NaOH solution, in order to exchange Cl⁻ with OH⁻. The resin was washed with water until the elution was neutral, then the hydrazino leucine was recovered by elution with 1N acetic acid. The fractions were evaporated to dryness and the crystalline solid was washed with cold ethanol.

Wt. of L-hydradino leucine, 0.150g (36.0%).
Preparation of Urea alcohol 44

To a solution of amino alcohol 29 (0.0288 g, 0.129 mmol) in water (3 mL) was added KOCN (0.0314 g, 0.387 mmol). The reaction mixture was stirred at 60°C for 12 h. TLC showed the reaction to be complete (50% MeOH/CHCl₃). The reaction mixture was freeze dried and the white solid, thus obtained, was washed with acetone. The extracts were filtered to remove insoluble matter, and concentrated in vacuo to afford 0.0264 g (76.9 %) of urea alcohol 44 as a yellow oil.

Urea Alcohol 44

1H NMR: 6.02 (1H, br.s), 5.15 (2H, br.s), 4.68 (1H, d; J=6.7 Hz), 4.63 (1H, d; J=6.7 Hz), 4.52 (1H, dd; J=6.3, 5.1 Hz), 3.76-3.50 (3H, br.), 3.40 (3H, s), 3.318 (3H, s), 3.315 (3H, s), 1.86 (2H, br.).

13C NMR: 159.9, 102.2, 97.4, 62.0, 56.0, 54.8, 53.1, 53.0, 35.4.

IR: 3355.9, 2937.5, 2851.2, 1748.8, 1662.5, 1616.0, 1549.6, 1476.6, 1383.6, 1131.2, 1038.3, 965.2, 918.8.


High Resolution MS: Expected Mass: 266.147750
Observed Mass: 266.14820
Urea alcohol 44
Urea alcohol 44
RuO$_2$ oxidation of TROC amino alcohol compound 46

To a solution of TROC amino alcohol 32 (0.0650 g, 0.163 mmol) in CCl$_4$ (2 mL) was added water (2 mL). NaIO$_4$ (0.174 g, 0.815 mmol) and RuO$_2$ (0.0033 g, 0.0244 mmol) were added to the reaction mixture. The mixture was stirred vigorously at room temperature for 24 h, after which time TLC showed completion (12 % MeOH/CHCl$_3$). Small amount of NaHSO$_3$ was added to the solution to destroy RuO$_4$. The reaction mixture was passed through a celite bed for removing RuO$_2$. The reaction mixture was extracted with EtOAc. For ease of characterization, the acid was converted to the methyl ester with CH$_2$N$_2$ and purified by silica gel chromatography.

Wt. of the ester 0.0346 g (50.0 %) as a yellow oil.
Troc amino methyl ester 46

$^1$H NMR: 6.48 (1H, br.d; J=8.4 Hz), 4.73 (2H, d; J=3.0 Hz), 4.68 (1H, d; J=7.2 Hz), 4.62 (1H, d; J=7.2 Hz), 4.53 (1H, dd; J=7.9, 4.6 Hz), 4.45 (1H, dd; J=8.5, 2.3 Hz), 3.96 (1H, ddd; J=8.7, 4.4, 2.5 Hz), 3.78 (3H, s), 3.44 (3H, s), 3.34 (3H, s), 3.31 (3H, s), 2.10-1.87 (2H, m).

IR: 3335.9, 2957.4, 2837.9, 1735.5, 1695.7, 1655.9, 1523.0, 1436.7, 1383.6, 1337.1, 1290.6, 1224.2, 1164.4, 1124.6, 1051.6, 985.2, 918.8.


High Resolution MS: Expected Mass: 425.041050
Observed Mass: 425.03902